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(54) [Title of Invention] Sample Injection Apparatus for Capillary Electrophoresis

(57) [Abstract]

[Problem]

To provide a sample injection apparatus for capillary electrophoresis with which a small amount of sample can be introduced efficiently.

[Means for Solving Problem]

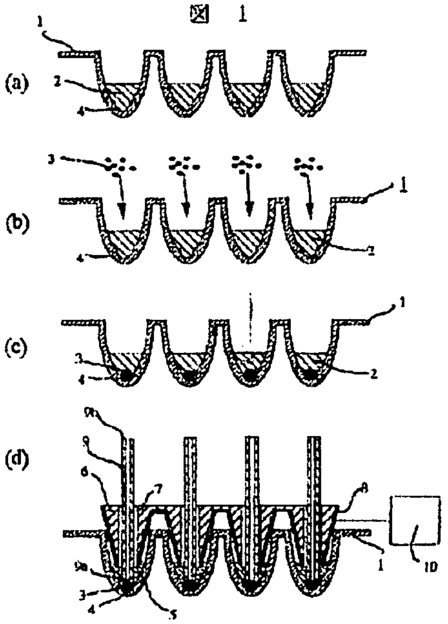
Sample molecules are induced to migrate under an electric field to near an electrode 8 disposed at capillary tip 9a in a solution, or microparticles 3 of an immobilized sample are collected, the sample is concentrated near the tip of the capillary, and the sample is injected into the capillary under an electric field.

[Effect]

It is possible to reduce the amount of sample and to reduce the cost of analysis.

Fig. 1

図 1



[Claims]

[Claim 1]

A sample injection apparatus for a capillary electrophoresis device wherein both ends of a capillary tube are immersed in a solution containing the respective electrolyte, voltage is applied between the two ends of the capillary tube inducing the sample to migrate under an electric field from one end to the other of the capillary tube, and the sample is separated into each component, said sample injection device characterized in that it has means for concentrating the sample near the end on the sample injection side of the capillary tube.

[Claim 2]

The sample injection apparatus according to claim 1, said sample injection apparatus characterized in that the means for concentrating the sample involves the adsorption of the sample on microparticles dispersed in a solution containing the sample and the collection of the microparticles near the end surface on the sample injection side.

[Claim 3]

The sample injection apparatus according to claim 2, said sample injection apparatus characterized in that once the precursor of a preliminary reaction has been immobilized on the microparticles and the product has been produced on the surface of the microparticles by reaction in the solution, the means for concentrating the sample involve collecting the microparticles near the end surface on the sample injection side.

[Claim 4]

The sample injection apparatus according to claim 1, said sample injection apparatus characterized in that means for concentrating said sample involve using an electrode disposed at the tip of the capillary tube and causing the sample to move under an electric field and become concentrated.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention]

The present invention pertains to a capillary electrophoresis apparatus, and in particular, provides a sample injection apparatus having a structure ideal for the analysis of a trace sample.

[0002]

[Prior Art]

In the past a sample has been injected into a capillary electrophoresis apparatus by the structure shown in Figure 4. In essence, one end 31a of a capillary 31 is introduced into an electrolyte solution 33 in which the sample has been dissolved, and the other end 31b is introduced into an electrolyte solution 34, electrodes 35 and 36 are introduced into solutions 33 and 34, respectively, voltage is applied between electrodes 35 and 36 by a power source 32, causing the sample to move under the potential gradient generated between end 31a of the capillary and electrode 35, and sample is injected inside the capillary. The sample molecules injected into the inside of the capillary are induced to migrate, under an electric field, through the inside of the capillary and are then detected by an absorption spectrometer using an ultraviolet lamp 37 and a detector 38 (for instance, *Biophysics*, **31**(1):30-33 (1991)).

[0003]

[Problems to Be Solved by the Invention]

By means of conventional methods, there is a problem in that a large volume of sample solution is needed when compared to the amount of sample that is actually injected into the capillary. For instance, when a capillary having an inner diameter of 0.1 mm is used, the volume of sample actually injected into the capillary is 20 or 30 nanoliters, but at least one microliter of solution is needed for the injection. Consequently, there is a problem in that injection is uneconomical because only 1/100th of the prepared sample solution is used for actual analysis.

[0004]

An object of the present invention is to provide a sample injection apparatus for capillary electrophoresis with which a small amount of sample can be efficiently introduced.

[0005]

[Means for Solving Problem]

The above-mentioned problem is associated with the position of the electrode and the capillary during sample injection. In essence, during sample injection, the tip of a capillary having an outer diameter of 0.2 mm (inner diameter) and the tip of an electrode made from a fine wire of Pt or similar material are simultaneously immersed in a sample solution and voltage is applied between the two. However, when a predetermined voltage is applied with good reproducibility, because of limitations in terms of mechanical precision, the dimension of one side becomes as much as 1 mm, in essence, so that 1 microliter of solution becomes necessary, and it is difficult to reduce the volume of liquid; in essence, it is difficult to conserve solution. The operation of simultaneous immersion and placement of the electrode and capillary in 20 or 30 nanoliters of solution is difficult because of these limitations to mechanical reliability, and in order to solve this problem, the sample is not prepared as a uniform solution; by means of the present invention, the sample is concentrated near the tip of the capillary.

[0006]

By means of the present invention, sample is immobilized on microparticles using microparticles, on the surface of which sample molecules can be immobilized, and then the microparticles are placed in the liquid, near the tip of the capillary. The sample can be eluted from the carrier by means of the effect of light, or the chemical effect of a buffer. The diffusion coefficient of sample molecules in the buffer is approximately 10^{-7*} cm^2/second in the case of a polymer such as DNA; therefore, the diffusion distance of sample molecules in a standing liquid at uniform temperature is only approximately 0.01 mm in 100 seconds, and is less than the outer dimension of the capillary. Consequently, if the sample is pre-concentrated near the tip of the capillary, the sample will not diffuse and will stagnate at a high concentration near the tip surface of the capillary, even if there is a large volume of liquid. Therefore, it is possible to efficiently inject the sample.

[0007] The sample injection apparatus for a capillary electrophoresis device wherein both ends of a capillary tube are immersed in a solution containing the respective electrolyte, voltage is applied between the two ends of the capillary tube inducing the sample to migrate under an electric field from one end to the other of the capillary tube, and the sample is separated into each component according to the present invention is designed such that it has means for concentrating the sample near the end on the sample injection side of the capillary tube, and the means for concentrating the sample is

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[*] This number is unclear in the Japanese document—Trans. note.

designed such that it involves the adsorption of the sample on microparticles dispersed in a solution containing the sample and the collection of the microparticles near the end surface on the sample injection side. The means for concentrating the sample is further designed such that an electrode disposed at the tip of the capillary tube is used to cause the sample to migrate under an electric field and become concentrated. The means for concentrating the sample is further designed such that once the precursor of a preliminary reaction has been immobilized on the microparticles and the product has been produced on the surface of the microparticles by reaction in the solution, the microparticles near the end surface on the sample injection side are collected.

[0008]

[Embodiments of the Invention]

(Working Example 1)

Figure 1 is a cross section showing the structure of the sample injection apparatus of the first working example. A reaction solution 2 is introduced into a vessel 1 and a reaction is performed using a reactor such as a thermal cycler (not illustrated). For instance, when analyzing the base sequence of DNA, a sequence reaction is performed using a biotinylated primer to produced a biotinylated DNA fragment (Figure 1(a)), microparticles 3 on which avidin has been deposited, such as magnetic beads, agarose particles, or glass particles, are introduced into vessel 1, and the biotinylated DNA fragment is immobilized using the specific binding between avidin and biotin (Figure 1(b)). By means of the process shown in Figures 1(a) and 1(b), it is also possible to pre-immobilize the primer and template DNA used in the sequence reaction on microparticles 3 and then to produce the DNA fragment on the surface of microparticles 3 by performing the sequence reaction. In essence, a precursor of the reaction is pre-immobilized on the microparticles, the product is produced on the surface of the microparticles by reaction in the solution, and the microparticles are collected near the end surface on the sample injection side. Next, the carrier is trapped in indentations 4 at the base of the vessel using a centrifuge, and reaction solution 2 is discarded (Figure 1(c)).

[0009]

Next, when a solution 5 containing electrolyte, such as a mixture of TBE buffer and formaldehyde, is introduced into each vessel, the bond between the avidin and the biotin is broken and the DNA is released into the solution. However, the diffusion coefficient of the DNA in the solution is small, and the DNA therefore stagnates near indentation 4 in the vessel. A support 6 made from an insulating material is inlaid in the vessel, and a capillary tube 9 is introduced from a hole 7 in the support. The hole in the support is aligned with indentation 4; therefore, a tip 9a of the capillary tube is positioned in indentation 4 where the DNA is present in a high concentration.

[0010]

In this state, a constant-voltage power source 10 applies voltage to an electrode 8 preformed in the surface of support 6. The amount of solution 5 is an amount that is sufficient for contact with electrode 8. A DNA sample has a negative charge; therefore, when negative voltage is applied to electrode 8 and the other end 9b of the capillary is brought to ground potential, DNA can be introduced inside the capillary (Figure 1(d)).

[0011]

By means of the first working example, the sample that is present in a diluted concentration in the reaction solution can be concentrated using microparticles and positioned at one end of the capillary. Therefore, a very efficient sample injection becomes possible. Moreover, by means of the present working examples, the reactor for preparing the sample uses an external vessel appropriate for standard reactors such as a microtiter plate, and this vessel can be used as the vessel for sample injection without being modified or replacing the vessel throughout any of the processes, from the reaction to the introduction of the sample into the capillary. Thus, the procedure is simplified.

[0012] (Second working example) Figure 2 is a cross section showing the structure of the sample injection apparatus of the second working example. Indentations 12 are made in a plate member 11, and a filter 13 is disposed in the base of indentation 12. Reaction solution 2 is introduced into indentations 12, the top and bottom are sandwiched between covers 14a and 14b, the unit is placed on a heat block 15 and a reaction is performed (Figure 2(a)). Once the reaction is completed, covers 14a and 14b are removed, microparticles 3 are introduced into each indentation, and the sample is immobilized (Figure 2(b)).

[0013] Next, plate member 11 is sandwiched between cover 14a and a member 16 and reaction solution 2 is discarded, suctioned from an opening 18. The mesh of the filter is smaller than the size of microparticles 3 and microparticles 3 can remain on the surface of filter 13 (Figure 2 (c)).

[0014] Next, plate member 11 on which microparticles 3 remain is carefully immersed in electrolyte solution 5, and the end surface 9a of capillary 9 is placed in each indentation 12. By applying negative potential to an electrode 19 using constant-voltage power source 10 with end surface 9b of the capillary at ground potential, the sample (DNA fragment) immobilized on microparticles 3 is injected inside capillary 9 (Figure 2(d)).

[0015] By means of the second working example, it is possible to collect more sample using many indentations 12 and filters 13 in high density. Moreover, it is also possible to use the filter of the present working example as a unit that directly adsorbs the sample molecules. In this case, it is possible to predeposit avidin on the filter or to delay the suction speed when reaction solution 2 is passed through the filter (Figure 2 (c)) in order to assure enough time for the immobilization reaction.

[0016] (Third working example) Figure 3 shows a cross section of the structure of the sample injection apparatus of the third working example. An electrode 21 is placed near end surface 9a of capillary tube 9 and is introduced into electrolyte solution 29 containing sample molecules. A separate electrode 22 is introduced into the same solution. An insulator 20 holds electrode 21. Insulator 20 is cylindrical, and the electrode is formed on the inside surface. The electrode can also be formed on the inside surface of a thin glass tube. First, voltage is applied between electrodes 19 and 22. When the sample is DNA, electrode 22 is brought to a ground potential 26 (connection between contacts 28a and 28b), and electrode 21 is brought to a positive potential (contacts 27a and 27b are connected; reference 23 is the positive-potential constant-voltage power source). The DNA molecules are concentrated around electrode 21 by this potential. Next, when electrodes 21 and 22 are brought to a negative potential (contacts 27a and 27c and contacts 28a and 28c are connected; references 24 and 25 indicate the negative-potential constant-voltage power source), DNA molecules are injected inside the capillary. By means of the above-mentioned series of operations, the potential of end surface 9b of the capillary is maintained at ground potential. Insulator 20 is the member that holds electrode 21 and is cylindrical, and electrode 21 is formed on the inside surface.

[0017] By means of the third working example, DNA can be concentrated to a high concentration around the end surface of the capillary tube using electrophoresis and the efficient injection of even a dilute solute becomes possible.

[0018]

[Effect of the Invention] By means of the present invention, it is possible to concentrate sample molecules near the tip of a capillary; therefore, efficient sample injection is possible. As a result, it is possible to reduce analysis cost by reducing the amount of sample analyzed.

[Brief Description of the Drawings]

[Figure 1] is a cross section showing the structure of the sample injection apparatus of the first working example.

[Figure 2] is a cross section showing the structure of the sample injection apparatus of the second working example.

[Figure 3] is a cross section showing the structure of the sample injection apparatus of the third working example.

[Figure 4] is a drawing showing the structure of an example of an apparatus of the prior art.

[List of reference numbers]

1 is a reactor, 2 is a reaction solution, 3 indicates microparticles, 4 is an indentation, 5 is an electrolyte solution, 6 is a support, 7 is a hole, 8 is an electrode, 9 is a capillary, 9a is the end surface on the sample injection side of the capillary, 9b is the other end surface of

the capillary, 10 is a constant-voltage power source, 11 is a plate member, 12 is an indentation, 13 is a filter, 14a and 14b are covers, 15 is a heat block, 16 is a member, 17 is the reaction solution that will be suctioned, 18 is an opening, 19 is an electrode, 20 is an insulator, 21 is an electrode, 22 is an electrode, 23 is a constant-voltage power source (positive potential), 24 is a constant-voltage power source (negative potential), 25 is a constant-voltage power source (negative potential), 26 is ground potential, 27a, b, and c are contacts, 28a, b, and c are contacts, 29 is an electrolyte solution containing sample molecules, 31 is a capillary, 31a is one end of the capillary, 31b is the other end of the capillary, 32 is a constant-voltage power source, 33 is an electrolyte solution (containing sample), 34 is an electrolyte solution, 35 is an electrode, 36 is an electrode, 37 is an ultraviolet lamp, and 38 is a detector.

Figure 1

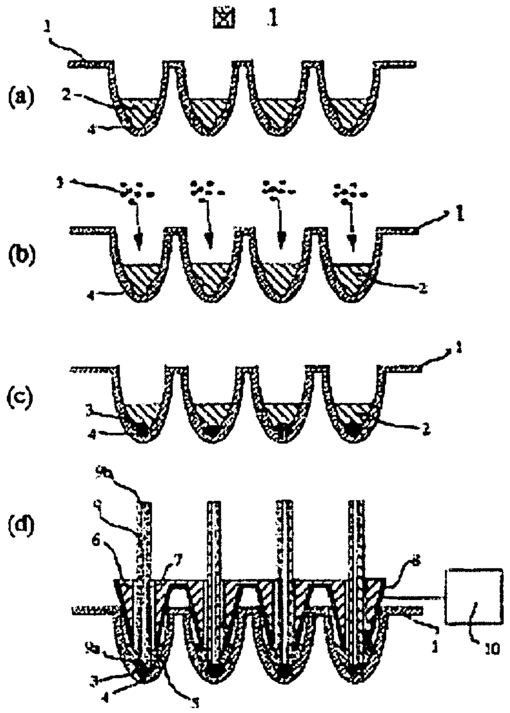

Figure 2

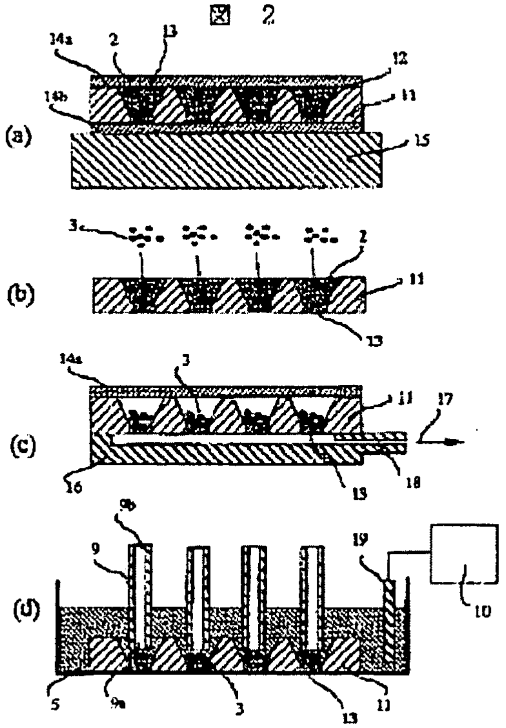

Figure 3

【図3】

図 3

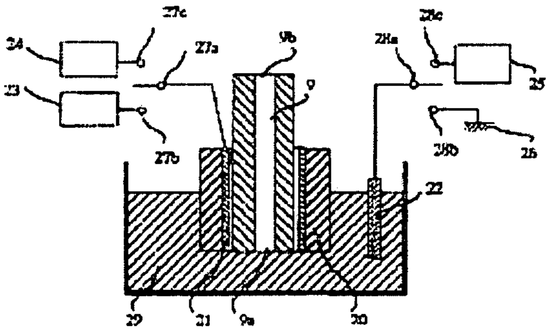

Figure 4

図 4